CHDR
Centre for Human Drug Research

SAB SYNOPSIS – CHDR2156

Title

Observational study to evaluate inter- and intra-subject variability of microcirculatory and endothelial functional tests and differences in these tests between several subject populations.

Short Title

Validation of microcirculatory and endothelial tests.

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Background & Rationale

The human body has several characteristics considering blood flow. An example of these characteristics is flow motion, which is defined as repetitive variations (oscillations) in the microcirculation.[1] Flow motion could correlate with the microcirculatory status of the vascular system in a specific region and whenever flow motion is not working properly, this could be linked to several diseases and disorders.[2–4] Moreover, flow motion could be used as indicator of tissue viability.[5] For this reason, new quantitative assessments might be useful to identify potential early symptoms of diseases and disorders or to review corresponding tissue viability. This current research will focus on several techniques to assess microcirculation and endothelial function, namely, Laser speckle contrast Imaging (LSCI), Flow Mediated Skin Fluorescence (FMSF), near infrared spectroscopy (NIRS), side-stream dark field microscopy (SDFM) and passive leg movement (PLM).

The aim of this non-interventional study is executing several noninvasive, simple, and reliable methods to evaluate inter- and intra-subject variability of microcirculatory and endothelial functional tests and differences in these tests between several subject populations. With use of FMSF, the nicotinamide adenine dinucleotide-reduced form (NADH) fluorescence intensity which is emitted from skin tissue on the forearm will be measured.[6,7] NADH fluorescence is characterized by its sensitivity towards the supply of oxygen, which is usually supplied to the epidermis by use of the skin microcirculation. Therefore, NADH fluorescence could be used as an indirect monitor of skin blood flow.[1] Nitric oxide dependent vasodilation will be assessed with local thermal hyperemia (LTH) challenges during LSCI, general skin microcirculatory and flow function will be assessed with occlusion-reperfusion (PORH) LSCI challenges. Additionally, muscle oxygen consumption and blood flow will be assessed with NIRS. Sublingual microvasculature and endothelial glycocalyx will be examined with SDFM, an emerging method developed to assess microvascular function. [8] Lastly, flow increase in the femoral artery induced by PLM will be measured to evaluate endothelial function. By measuring these endpoints, this study allows a more thorough characterization of this ‘endothelial test battery’ for further use in interventional clinical trials.

Strategic relevance

CHDR currently has several techniques to measure microvasculature and endothelial function as listed above. By measuring these endpoints multiple times in various populations, this study allows a more thorough characterization of this ‘endothelial test battery’ for further use in interventional clinical trials.

Operational considerations

Due to the non-WMO nature of this study, the study can be conducted with relatively little investment of CHDR resources. Subjects will be able to contact the CHDR and will be scheduled for an appointment with a CHDR intern conducting the endothelial measurements. On the first study day, a physician will conduct a simple screening after which subjects can be included and measured by the CHDR intern.

Objective(s)

Primary Objectives

- To assess inter- and intrasubject variability of tests of endothelial function and microcirculation.

Secondary Objectives

To compare

- Microcirculation and endothelial function in young healthy volunteers (age 18-30) to old healthy volunteers (age 45-75).
- Microcirculation and endothelial function in young healthy volunteers (age 18-30) to patients with mitochondrial function.

Design

Eight healthy volunteers will be assessed in this study on two separate study days. The Screening Visit will be conducted at day 1. An overview of the assessments is provided in the visit and assessment schedule (Table 1). Each study day subjects will undergo a battery of endothelial testing, which consists of the following measurements: Flow Mediated Skin Fluorescence (FMSF), laser speckle contrast imaging (LSCI), near infrared spectroscopy (NIRS), side-stream dark field microscopy (SDFM) and passive leg movement (PLM), in no particular order. However, LSCI_LTH measurements will be performed after LSCI_PORH.

Subjects / Groups

Group 1: N=8 young healthy volunteers, age 18-30

Group 2: N=8 subjects with confirmed mitochondrial disorder, specifically Friedreich's Ataxia (FRDA) = called MitoD subjects. All these subjects will also have an LVEF <50%. Data from these subjects are derived from the CHDR2111 study.

Group 3: N=8 old healthy volunteers (age 45-75). Data from these subjects are derived from CHDR2111 study.

Inclusion criteria (group 1)

1. Male and females, 18 to 30 years of age, inclusive.
2. Subject has voluntarily signed informed consent form.
3. Willingness and ability to comply with all study procedures
4. Body mass index (BMI) between 18 and 30 kg/m2

Exclusion criteria (group 1)

1. Evidence of any active or chronic disease or condition that could interfere with the conduct of the study (following a detailed medical history, physical examination, vital signs (systolic and diastolic blood pressure, pulse rate, body temperature).
2. Receival of any study drug within 30 days or 5 times ½ half-life, whichever greater prior to day 1.

3. Participation in an investigational drug or device study (last dosing of previous study was within 90 days prior to first dosing of this study).
4. History of abuse of addictive substances (alcohol, illegal substances) or current use of more than 21 units alcohol per week, drug abuse, or regular user of sedatives, hypnotics, tranquillisers, or any other addictive agent.
5. Alcohol intake 24h prior to the study day.
6. Is demonstrating excess in caffeine consumption (more than eight cups of coffee or equivalent per day.
7. If a woman, pregnant, or breast-feeding, or planning to become pregnant during the study
8. Any daily nicotine use or regular use of nicotine products.
9. Positive nasopharyngeal rapid antigen test for SARS-CoV-2 at admission to the clinical research center
10. Subject has received any vaccination in the last 2 weeks prior to Visit 1

Concomitant medications

For all subjects, all concomitant medication will be documented at Screening and evaluated by the investigator. For healthy volunteers, no use of any form of medication will be allowed within 1 months of start of study. Exceptions are paracetamol up to 4g/day and ibuprofen up to 1g/day. Other exceptions will be made at the discretion of the investigator and clearly documented.

Efficacy endpoints

Objective	Assessment	Endpoints
To assess inter- and intrasubject variability of various tests of endothelial function and microcirculation. To compare microcirculation and endothelial function in young healthy volunteers (age 18-30) to old healthy volunteers (age 45-75). To compare microcirculation and endothelial function in young healthy volunteers (age 18-30) to patients with mitochondrial function.	**Near-infrared spectrometry (NIRS).**	• Blood flow measured as slope of hemoglobin increase in the arm during venous occlusion • Oxygen consumption measured as desaturation slope during arterial occlusion • Reactive hyperemia measured as saturation slope after arterial occlusion • Duration of reactive hyperemia measured as time to return to baseline after arterial occlusion
	Laser speckle contrast imaging (LSCI).	• Basal flow before LTH and PORH • Peak flow after arterial occlusion • Peak flow during thermal hyperemia • Plateau flow during thermal hyperemia
	Side-stream darkfield microscopy (SDFM).	• Number of vessels • Vessel density • Perfused number of vessels • Perfused vessel density • Percentage perfused vessels

	Passive Leg Movement (PLM).	• Flow (mL/s) before leg movement • Peak flow (mL/s) after leg movement • Flow change (mL/s) • Area under the curve of flow graph, i.e. total flow (mL)
	Flow Mediated Skin Fluorescence (FMSF).	• Ischemic response • Hyperemic response • Baseline, min, max and post-test NADH fluorescence • Vasomotion parameters

Sample Size Justification

This is an observational case-control study to compare microcirculation and endothelial function in several patient groups with healthy volunteers. The distribution and variability of these factors are unknown in these populations; therefore, the sample size is selected to obtain an exploratory sample and not based on statistical considerations.

Statistical methodology

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. All endpoints will be summarized by cohort using descriptive statistics.

REFERENCES

[1] Katarzynska J, Cholewinski T, Sieron L, Marcinek A, Gebicki J. Flowmotion Monitored by Flow Mediated Skin Fluorescence (FMSF): A Tool for Characterization of Microcirculatory Status. Frontiers in Physiology 2020;11. https://doi.org/10.3389/FPHYS.2020.00702.

[2] Sorelli M, Francia P, Bocchi L, de Bellis A, Anichini R. Assessment of cutaneous microcirculation by laser Doppler flowmetry in type 1 diabetes. Microvascular Research 2019;124:91–6. https://doi.org/10.1016/J.MVR.2019.04.002.

[3] Bari F, Tóth-Szuki V, Domoki F, Kálmán J. Flow motion pattern differences in the forehead and forearm skin: Age-dependent alterations are not specific for Alzheimer's disease. Microvascular Research 2005;70:121–8. https://doi.org/10.1016/J.MVR.2005.09.001.

[4] Schmidt-Lucke C, Borgström P, Schmidt-Lucke JA. Low frequency flowmotion/(vasomotion) during patho-physiological conditions. Life Sciences 2002;71:2713–28. https://doi.org/10.1016/S0024-3205(02)02110-0.

[5] Heeman Wiendelt Steenbergen Gooitzen van Dam E Christiaan Boerma Wido Heeman WM, Steenbergen W, van Dam GM, Christiaan Boerma E, Heeman W. Clinical applications of laser speckle contrast imaging: a review. Https://DoiOrg/101117/1JBO248080901 2019;24:080901. https://doi.org/10.1117/1.JBO.24.8.080901.

[6] Piotrowski L, Urbaniak M, Jedrzejczak B, Marcinek A, Gebicki J. Note: Flow mediated skin fluorescence—A novel technique for evaluation of cutaneous microcirculation. Review of Scientific Instruments 2016;87:036111. https://doi.org/10.1063/1.4945044.

[7] Bogaczewicz J, Tokarska K, Wozniacka A. Changes of NADH Fluorescence from the Skin of Patients with Systemic Lupus Erythematosus. BioMed Research International 2019;2019. https://doi.org/10.1155/2019/5897487.

[8] Djaberi R, Schuijf JD, de Koning EJ, Wijewickrama DC, Pereira AM, Smit JW, et al. Non-invasive assessment of microcirculation by sidestream dark field imaging as a marker of coronary artery disease in diabetes. Diabetes & Vascular Disease Research 2013;10:123–34. https://doi.org/10.1177/1479164112446302.

Table 1 Visit and Assessment Schedule

Time point / Assessment	SCR	Study Day 1	Study day 2[1]
Informed consent	X		
SARS-CoV-2 Antigen test	X		X
Demography	X		
Inclusion and exclusion criteria	X		
Medical history	X		
Physical examination	X		
Concomitant medication	X		
Meals / snack		X	X
Temperature	X		
Vital Signs (HR, BP)	X		
FMSF[2]		X	X
PLM[2]		X	X
SDFM[2]		X	X
LCSI LTH[2]		X	X
LSCI PORH[2]		X	X
NIRS[2]		X	X

HR=Heart Rate, BP=Blood Pressure, SCR=Screening, ECG=Electrocardiography, SCR=Screen, FMSF=Flow Mediated Skin Fluorescence, PLM=Passive Leg Movement, SDFM=Side-stream darkfield microscopy, LSCI=Laser Speckle Contrast Imaging, LTH=Local Thermal Hyperemia, PORH=Post-Occlusive Reactive Hyperemia, NIRS=Near infrared spectroscopy

[1] Study day 2 will take place at least 5 days after study day 1
[2] Measurements will be performed in any order. However, LSCI_PORH and LSCI_LTH will be executed sequentially in that order.